Use of the Dominant-Lethal Test to Detect Genetic Activity of Environmental Chemicals

by Samuel S. Epstein*

Genetic Basis of the Dominant-Lethal Test

Potential genetic hazards due to drugs and chemical pollutants are now generally recognized. Such recognition has been paralleled by the recent development of appropriate sensitive, and practical *in vivo* mammalian methods for detecting and measuring mutagenic effects due to chemicals (*1,2*). These methods have a high degree of presumptive human relevance and include *in vivo* cytogenetics, the host-mediated assay, and the dominant-lethal assay; submammalian systems are generally considered to yield data of ancillary value (*3*). An expert HEW committee has recently recommended that drugs, pesticides, food additives and other synthetic chemicals be tested for mutagenicity in mammalian systems prior to their use or registration (*1*).

Dominant-lethal mutations have been used as convenient indicators of major genetic damage for measuring the effects of x-ray and of mutagenic chemicals in mammals. Such data may be appropriately extrapolated to man, especially as many recognizable human autosomal traits are due to dominant mutations (*4*).

The genetic basis for dominant lethality is mainly the induction of structural and numerical chromosomal anomalies, such as translocations and aneuploidies (Table 1); sequentially, these may induce preimplantation losses of nonviable zygotes, early fetal deaths, and sterility and semisterility in F_1 progeny (*5–9*). Translocations have been identified in F_1 progeny of mutagen-treated male rodents (*7–11*) and also in human abortions and congenital malformations (*12*).

Table 1. Genetic basis of the dominant test.

Presumed genetic basis	Associated genetic anomalies
Aneuploidy	Sterility and semisterility
Deficiencies: unhealed chromosome breaks	Viable reciprocal translocations in F_1 progeny
Reciprocal translocations	Cytogenetic effects in early cleavage and live embryos

The Dominant-Lethal Test in Relation to Standard Toxicological Practice

Assays should be conducted on both mice and rats as standard toxicological practice. Species variation in sensitivity has already been noted (*13*); for example, DDT does not induce either preimplantation losses or early fetal deaths in mice (S. S. Epstein, unpublished data) but does so in rats (M. Legator, unpublished data). Chemicals should be tested for mutagenicity after acute, subacute

*Swetland Professor of Environmental Health and Human Ecology, Case Western Reserve University Medical School, Cleveland, Ohio 44106.

Table 2. Recommended scheme for dominant-lethal testing in male mice (*14*).

	Administration route	Dose schedule	Dose level	Animals[1]
Acute	Oral or parenteral	Single dose	0.2 LD_{50}	Male mice mated with 3 females each for 5 successive weeks
Subacute	Oral or parenteral	Daily for 5 days	0.2 LD_{50}	Male mice mated with 3 females each for 5 successive weeks
Chronic	Oral	Daily for 3 months	Maximally tolerated dose	Male mice mated with 3 females each for 4 successive weeks

and chronic administration to male rodents by oral, parenteral, and respiratory routes (Table 2).

The route of test should reflect human exposure. Subacute testing is recommended largely to anticipate and reflect the role of possible hepatic microsomal detoxification or activation, while the object of chronic administration is to detect cumulative spermatogonial mutations. Enough animals must be used so that a test which indicates differences significant at the 5% level will have a 99% probability of detecting any true difference that exceeds 20% of the control mean number of living implants. For initial testing, a single dose is adequate. In acute and subacute testing, 0.2 of the LD_{50}'s are recommended, while maximally tolerated doses are appropriate for chronic testing (*14*).

In vivo mammalian tests for mutagenicity could be practically and economically integrated in routine toxicity testing. In acute, subacute and chronic toxicity testing, for example, or in the course of carcinogenicity tests, male mice or rats may be mated with untreated females which can subsequently be examined for the induction of dominant lethal mutations, indicated by early fetal deaths and preimplantation losses. Cytogenetic analyses in rats can be performed serially on peripheral blood, on bone marrow, and possibly on testes at death as a standard procedure in toxicity testing. In this context, it has recently been suggested that testing for toxicity, teratogenicity, carcinogenicity and mutagenicity could be performed on the same groups of animals (*15*). Finally, in common with all toxicological procedures, the protocols recommended here should dynamically reflect technical and conceptual advances.

The "Conventional" Dominant-Lethal Test

In the conventional dominant-lethal assay (*8, 16*), male mice or rats are dosed singly with subtoxic concentrations of the drugs to be tested. They are then mated sequentially with groups of untreated females. Matings in weeks 1–2, 3–4, and 5–8 after treatment of male mice represent samples of postmeiotic, meiotic, and premeiotic stages of spermatogenesis, respectively; corresponding periods in the rat are weeks 1–4, 5–6, and 7–10. Timing of stage sensitivity can be complicated by delayed metabolic activation or detoxification of the drug or by drug-induced inhibition of mitosis or meioses. Females are inspected daily for vaginal plugs, dissected on approximately the fourteenth day of pregnancy, and scored for corpora lutea and for total implants comprising early and late fetal deaths and living foetuses. Mutagenic effects are expressed conventionally as the mutagenic index (early fetal deaths/total implants) × 100.

The "Modified" Dominant-Lethal Test

The dominant-lethal assay has recently been modified and simplified for large-scale

routine testing in mice (*5, 12, 16–18*). Inspection for vaginal plugs is omitted; instead, all females are dissected 13 days after the midweek of their caging and presumptive mating. Corpora lutea counts, which are difficult and relatively imprecise in mice, are also omitted, and preimplantation losses are scored by contrasting values of total implants in females mated with treated and control males. Use of the modified assay may be recommended for routine screening in mice. In rats, however, matings should be timed and corpora lutea counted.

Detailed characterization of the reproductive parameters of large control populations is essential to the development of standard protocols for the dominant lethal assay (*18*). Such studies will define the range and cyclic variation in total implants, preimplantation losses, and early and late fetal deaths. Closed-colony, random-bred rodents are suitable for these assays, and the use of F_1 hybrids may minimize variation.

Scoring for Results in the Dominant-Lethal Test

Complete autopsy of females is essential, as intercurrent infection in any animal can induce preimplantation losses and early fetal deaths (*19*). The induction of dominant-lethal mutations is scored directly by an increased incidence of early fetal deaths and indirectly by an increased incidence of preimplantation losses, measured by the difference between total implants in control and test females, and/or by the difference between corpora lutea counts and number of total implants. Mutagenic effects may be reported directly in terms of the mutagenic index and/or as the number of early fetal deaths per pregnant mouse or indirectly in terms of preimplantation losses. As numerator and denominator are contributory to the mutagenic index, estimates of standard deviation are complex.

Initial testing may reasonably be restricted to meiotic and postmeiotic stages, for no chemical has been shown to induce dominant-lethal mutations exclusively in premeiotic stages. While various chemicals induce premeiotic effects, as measured by early fetal deaths and/or preimplantation losses (*5, 7, 8*), these also produce marked meiotic and/or post-meiotic effects. Mutagenic effects in acute, subacute, or chronic testing, as measured directly by increased early fetal deaths and/or indirectly by preimplantation losses, should be confirmed by subsequent testing *inter alia* over extended dose ranges. The dominant-lethal assay can also be used to investigate the possibility of synergistic, antagonistic or other interactive mutagenic effects; for example, caffeine is nonmutagenic and does not synergize the mutagenic effects of x-rays or chemical mutagens in mice (*20*).

Recent Experience with the Modified Dominant Lethal Assay

A total of 174 test agents, including pharmaceuticals, food additives, pesticides, and organic extracts of air and water pollutants, have recently been tested for mutagenicity in mice using the modified dominant-lethal assay (*18*). In concurrent control populations, the mean weekly pregnancy rate was 66% and exceeded 30% in 99% of all weeks; the distribution of weekly mean total implants per pregnancy was symmetrical around a peak of 11.5–11.9 and was never less than 8; mean early fetal deaths per pregnancy were 0.95 in 99.6% of weeks, and their distribution was highly asymmetrical. These parameters afforded the basis for screening the large body of test data (*18*).

All agents with mean weekly values beyond control limits were subject to analysis of variance for test and concurrent control data in all replicate experiments. Less than 10% of all agents tested were unequivocally mutagenic as determined directly by increased early fetal deaths per pregnancy and, in some instances, also indirectly by reduction in total implants per pregnancy; the majority of these are known alkylating agents. Additionally, about 5% of all agents tested yielded data which fell beyond con-

trol limits and which were significant at 5% by analysis of variance, but which, however, require further replication because of internal inconsistencies (*18*).

Essential Stages in the Development of the Dominant-Lethal Assay

Prior to the use of the dominant-lethal assay for screening for mutagenic effects, it is essential that careful and prolonged study and characterization of the reproductive parameters of the test rodent colony be undertaken. The range and pattern of cyclic and seasonal variation in incidence of pregnancies, numbers of total implants, preimplantation losses, and early and late fetal deaths in control animals must be statistically analyzed. Quality control limits must then be defined which establish limits of control ranges. On the basis of these limits, standard criteria may be developed for mutagenic activity of compounds under test (*18*). Data for any test agents meeting these standard criteria for mutagenicity should then be subject to analysis of variance and examined for internal consistency. Positive results should be confirmed by replication and by testing over extended dose ranges (*18*). Failure to recognize the critical importance of these problems may well result in experimentally simplistic approaches to the dominant-lethal assay and the development of inadequate and unreliable data, particularly false positive results.

REFERENCES

1. Advisory Panel on Mutagenicity of Pesticides, 1969. Report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health. U.S. Dept. of Health, Education, and Welfare, G.P.O., pp. 565–653.
2. Report of the NIEHS Task Force on Research Planning in Environmental Health Science. 1970. U.S. Dept. of Health, Education, and Welfare, G.P.O., pp. 158–161.
3. Friedman, L., et al. Food and Drug Administration, Advisory Committee on Protocols for Safety Evaluation, Panel on Reproduction. Report on reproduction studies in the safety evaluation of food additives and pesticide residues. Toxicol. Appl. Pharmacol. **16**: 264 (1970).
4. Report of the United Nations Scientific Committee on the Effects of Atomic Radiation. United Nations, New York, 1966, p. 99.
5. Epstein, S. S., et al. Mutagenic and antifertility effects of TEPA and METEPA in mice. Toxicol. Appl. Pharmacol. **17**: 23 (1971).
6. Joshi, S. R., et al. Fertilization and early embryonic development subsequent to mating with TEPA.-treated male mice. Genetics **65**: 483 (1970).
7. Rohrborn, G. The dominant lethals: Method and cytogenetic examination of early cleavage stages. In: Chemical Mutagenesis in Mammals and Man, F. Vogel and G. Rohrborn, Eds., Springer-Verlag, Berlin–New York, 1970, pp. 148–155.
8. Bateman, A. J., and Epstein, S. S. Dominant lethal mutations in mammals. In: Chemical Mutagens: Principles and Methods for Their Detection, A. Hollaender, Ed., Vol. 2, Plenum Press, New York, 1971, pp. 541–568.
9. Epstein, S. S., et al. Sterility and semisterility in male progeny of male mice treated with the chemical mutagen TEPA. Toxicol. Appl. Pharmacol. **19**: 134 (1971).
10. Cattanach, B. M., Pollard, C. E., and Isaacson, J. H. Ethylmethanesulfonate-induced chromosome breakage in the mouse. Mutation Res. **6**: 297 (1968).
11. Ford, C. E., et al. Differential transmission of translocations induced in spermatogonia by irradiation. Cytogenetics **8**: 447 (1969).
12. Carr, D. H. Chromosomal errors and development. Amer. J. Obstet. Gynecol. **104**: 327 (1969).
13. Generoso, W. M., and Russell, W. L. Strain and sex variations in the sensitivity of mice to dominant-lethal induction with ethylmethanesulfonate. Abstr. 38th Annual Meeting Genetics Society of America, Madison, Wisconsin, Aug. 18–20, 1968.
14. Epstein, S. S., and Rohrborn, G. Recommended procedures for testing genetic hazards from chemicals, based on the induction of dominant lethal mutations in mammals. *Nature* **230**: 459 (1971).
15. Epstein, S. S. A catch-all toxicological screen. Experientia **25**: 617 (1969).
16. Epstein, S. S., and Shafner, H. Chemical mutagens in the human environment. Nature **219**: 385 (1968).
17. Epstein, S. S., et al. Mutagenicity of trimethylphosphate in mice. Science **168**: 584 (1970).
18. Epstein, S. S., et al. Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicol. Appl. Pharmacol. **23**: 288 (1972).
19. Rohrborn, G. Mutagenicity tests in mice. Humangenetik **6**: 345 (1968).
20. Epstein, S. S., et al. The failure of caffeine to induce mutagenic effects or to synergize the effects of known mutagens in mice. Food Cosmet. Toxicol. **8**: 381 (1970).